

BAG3-related myofibrillar myopathy: a further observation with cardiomyopathy at onset in pediatric age

Gaia Scarpini¹, Maria Lucia Valentino^{2,3}, Melania Giannotta¹, Luca Ragni⁴, Annalaura Torella⁵, Marta Columbaro⁶, Vincenzo Nigro⁵, Antonella Pini¹

¹ Neuromuscular Pediatric Unit, UOC di Neuropsichiatria dell'età pediatrica, IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy; ² UOC di Clinica Neurologica, IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy; ³ Department of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy; ⁴ Pediatric Cardiology, University of Bologna, Bologna, Italy; ⁵ Telethon Institute of Genetics and Medicine (TIGEM), Università della Campania "Luigi Vanvitelli", Pozzuoli, Naples, Italy; ⁶ SC Musculoskeletal Cell Biology Laboratory, IRCCS Istituto Ortopedico Rizzoli, Bologna, Bologna, Italy

Myofibrillar myopathies are a heterogeneous group of neuromuscular disorders characterized by degeneration of Z-disk, causing the disintegration of myofibrils. They may be caused by mutations in different genes, among these, the BAG3 gene (Bcl-2 associated-athano gene-3) encodes a multidomain protein that plays an important role in many cellular processes. We report the case of a 16-year-old male who at 4 years of age presented with a hypertrophic obstructive cardiomyopathy, then developed axonal sensory motor polyneuropathy, muscle weakness, rigid spine, severe kyphoscoliosis and respiratory failure. Muscle biopsy showed the typical hallmark of myofibrillar myopathy with abnormal cytoplasmic expression of multiple proteins. A de novo heterozygous common mutation in the BAG3 gene with a c.626C > T (p.Pro209Leu) was discovered on NGS genetic analysis. Mutations in the BAG3 gene are causes of a severe and progressive condition and natural history data are important to be collected. An early diagnosis is critical for prognostic implications in cardiomyopathy and respiratory failure treatment.

Key words: BAG3, myofibrillar myopathy, cardiomyopathy, pediatric neuromuscular disorder

Introduction

Myofibrillar myopathies (MFM) are a heterogeneous group of neuromuscular disorders caused by mutations of different genes that share myopathological features, first of all the disintegration of Z-disks followed by myofibrillar disruption and ectopic accumulation of multiple proteins¹. Each gene produces a mutated protein which is an integral part of the Z-disk or is closely associated with it. The clinical manifestations of various subtypes can change and may include different age of onset (from childhood to late adulthood) and distal more than proximal weakness, cardiomyopathy, respiratory failure, cataracts or peripheral neuropathy in various

Received: October 27, 2021
Accepted: December 13, 2021

Correspondence

Antonella Pini

UOC pediatric Neurology and Psychiatry, IRCCS – Istituto delle Scienze Neurologiche di Bologna, via Altura 3, 40139 Bologna, Italy. Tel.: +39 051 6225111
E-mail: antonella.pini@isnb.it

How to cite this article: Scarpini G, Valentino ML, Giannotta M, et al. BAG3-related myofibrillar myopathy: a further observation with cardiomyopathy at onset in pediatric age. Acta Myol 2021;40:177-183. <https://doi.org/10.36185/2532-1900-061>

© Gaetano Conte Academy - Mediterranean Society of Myology



OPEN ACCESS

This is an open access article distributed in accordance with the CC-BY-NC-ND (Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International) license. The article can be used by giving appropriate credit and mentioning the license, but only for non-commercial purposes and only in the original version. For further information: <https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>

combinations. The diagnosis of MFM is based on clinical findings, electromyography, nerve conduction studies and muscle histology. The most frequent inheritance pattern of the MFM-causing genes is autosomal dominant, and in a significant number of patients the mutation occurs *de novo*²⁻³.

An early-onset subtype of MFM is caused by a mutation in *BAG3* gene on chromosome 10, encoding for the antiapoptotic Bag3 (Bcl-2 associated-athanogene-3) protein. Bag3 is a multi-domain protein that regulates the Hsp70 family of molecular chaperones and that interacts with many other polypeptides, strongly expressed in skeletal and cardiac muscle and at lower level in other tissues. Bag3 is involved in a panoply of cellular processes such as development, apoptosis, autophagy, cytoskeleton organization, cell adhesion and motility⁴.

The clinical presentation of *BAG3*-related MFM is usually characterized by limb and axial muscle weakness, peripheral neuropathy, cardiomyopathy and respiratory failure. In these patients the mutation P209L re-occur at a high frequency. In the typical presentation of MFM due to *BAG3* mutation, childhood cases can be particularly severe with rapid progression of the clinical picture and death in early adolescence⁵⁻⁷.

With the aim of contributing to better defining the natural history of *BAG3*-related MFM, we report here a Caucasian 16-year-old male patient with the p.Pro209Leu (c.626C > T) in exon 3 mutation and cardiomyopathy as first clinical sign associated with peripheral neuropathy and MFM due to *BAG3* mutation.

Case report

The 16-year-old Caucasian male had a negative family history for neurological disease and normal physiological history. Developmental milestones were normal. At 4 years a heart murmur was discovered and cardiologic examinations led to a diagnosis of hypertrophic obstructive cardiomyopathy (HOC). He started therapy with metoprolol. At 8 years, he was hospitalized for fatigability and gait abnormalities. Increased creatine kinase (583 U/L) was detected, and HOC was stable. A diagnosis of myositis was made. The symptomatology then improved, but he was referred to the Pediatric Neuromuscular Clinic for associated mildly clumsy gait. Genetic analysis for Friedreich's ataxia was negative. Neurological and neuromuscular examination at the age of 10 showed mild girdle and distal lower limb weakness (deltoids, biceps and triceps brachialis 4/5, tibialis anterior e peroneal muscles 4/5), pes cavus, rigid spine and ankle contractures with toe walking, positive Gower's sign and absence of deep tendon reflexes in upper and lower limbs. Romberg maneuver was positive. Creatine kinase was

increased (843 U/L). Nerve conduction study showed a severe sensory-motor axonal polyneuropathy with upper limbs motor conduction median nerve 37.5 m/s and ulnar nerve 40 m/s and slowed F wave. In the lower limbs, the compound motor action potential and the sensory action potential were not evocable.

Muscle biopsy revealed, on the hematoxylin and eosin staining, myopathic changes with marked fiber size variability, atrophic fibers occasionally angulated, abundant centrally placed nuclei, necrosis and mild increase in connective tissue (Fig. 1A). Type I fibers were predominant. Multiple small vacuoles were present in numerous fibers (asterisks, Fig. 1A) and eosinophilic areas were observed in the cytoplasm of many fibers (arrows, Fig. 1A), the same strongly reactive with the Gomori trichrome staining (arrows, Fig. 1B). Immunofluorescence analyses revealed ectopic expression of different sarcomeric protein like such as alphaB-crystallin (Fig. 1C) and myotilin (Fig. 1D).

Ultrastructural analysis confirmed the presence of severe signs of myofibrillar disruption with accumulation of electron dense granulo-filamentous materials in the intermyofibrillar space (Fig. 1E, Z-disk streaming, Fig. 1F) and abnormal extension of electron dense Z-bands (Fig. 1G).

Genetic analysis

LMNA, Desmin, alfaB-crystallin, myotilin e LDB3 genes didn't show mutations. We performed the analysis of an NGS panel that include 169 genes associated with neuromuscular conditions (MotorPlex) 8. Coverage was at least 50x for > 98% of target. We also included parents in the NGS study, and we discovered a single heterozygous mutation p.Pro209Leu (c.626C > T NM_004281.4) in exon 3 of *BAG 3* gene. This variant is currently not listed in gnomAD, and it is predicted to be pathogenic according to the ACMG/AMP criteria confirming the diagnosis of *BAG3*-myopathy. It was a *de novo* mutation, absent in the parents.

At 4 years the electrocardiogram showed left atrial enlargement and left ventricle hypertrophy, while the echocardiogram showed hypertrophic obstructive cardiomyopathy with a peak left ventricular outflow tract (LVOT) gradient of 50 mmHg; the interventricular septum thickness was 16 mm, the middle septum 17 mm, the posterior basal septum and the anterior basal septum 18 mm. The systolic function was normal (left ventricle ejection fraction, EF: 50%), while in diastole the left ventricle had high filling pressure. Subsequently, during the follow-up period, the features of the cardiomyopathy changed, the LVOT obstruction disappeared, but the restrictive pattern worsened with increase in the filling pressure of the left ventricle. The cardiac catheterization showed slight post-

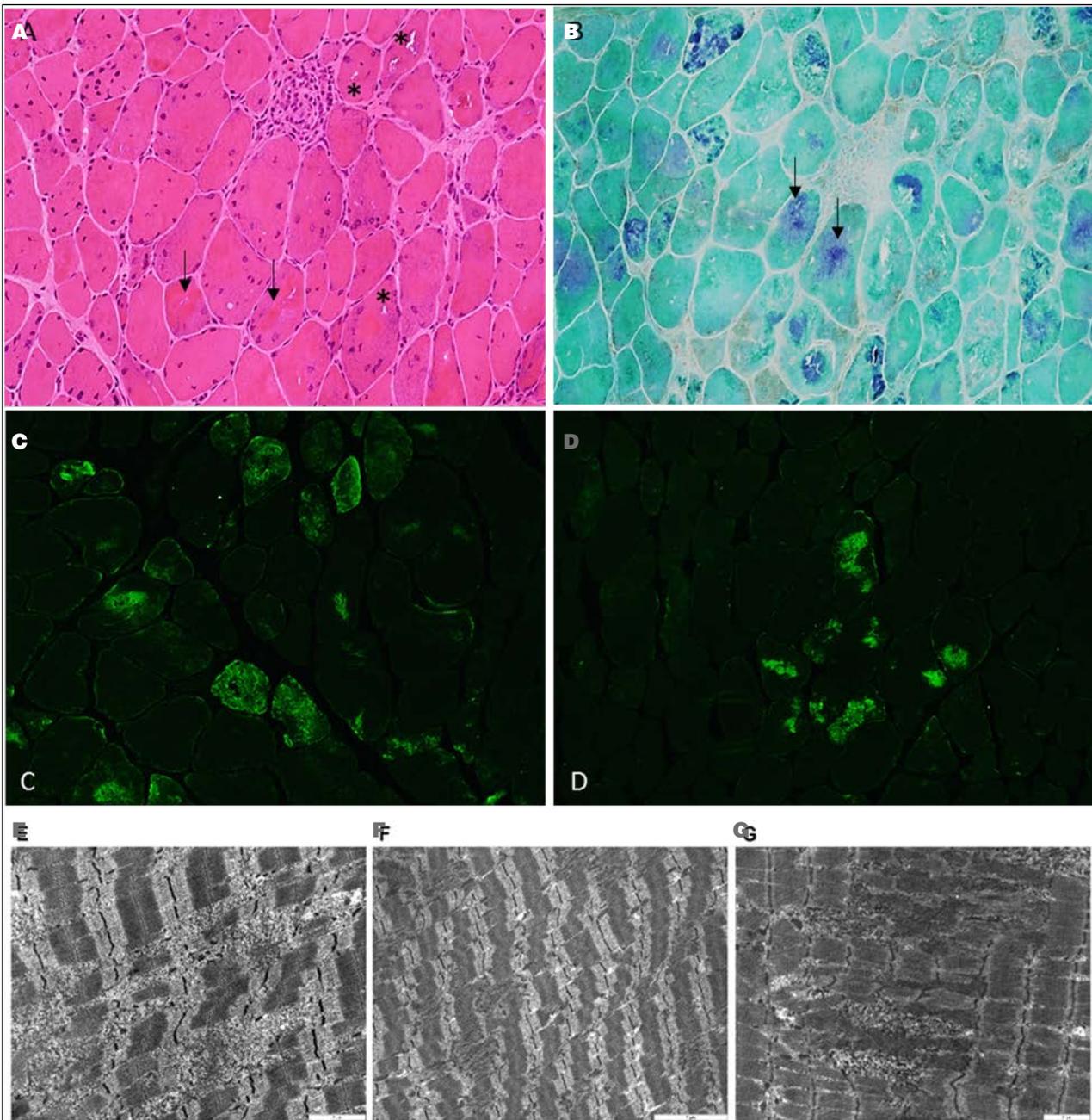


Figure 1. Morphological analyses of patient skeletal muscle tissue. **A)** hematoxylin and eosin (H&E) histological staining: protein inclusions and vacuoles indicated by arrows and asterisks, respectively (magnification 20x); **B)** trichrome Gomori staining: protein inclusions indicated by arrows (magnification 20x); **C,D)** immunofluorescence analyses with anti-alpha B crystallin(1C) and anti-myotilin (1D) antibodies showed protein aggregates inside the fibers (magnification 10x); **E-G)** ultrastructural analyses with Transmission Electron Microscopy (TEM) showed abnormal myofibrillar structures and Z-disk streaming.

capillary pulmonary hypertension with mild increase in vascular pulmonary resistance.

At 11 years hypercapnic restrictive respiratory failure (paCO₂ 84 mmHg) occurred. Hospitalization in the

Cardiological Intensive Care Unit and pulmonary assessment were necessary. Since then he needed non-invasive ventilation (NIV), first only at night, then also all day. Idebnone 600 mg/day was added to the therapy.

At 16 years, standing is possible with support and he is able to walk with support for a few minutes. Kyphoscoliosis is very severe. He becomes tired very easily. The weight is 33 kilos. The EF of the left ventricle is 55%. Dysphagia is not present and speech is fluent. He presented an episode of acute congestive heart failure which was treated till the remission of the acute phase. His cognitive performances are very good.

Discussion

BAG3-related MFM is a rare condition, in most cases due to a *de novo* mutation, and causes severe symptoms with rapidly progressive muscle, nerve, respiratory and heart involvement. The heterozygous mutation p.Pro209Leu (c.626C > T) has previously been identified in several patients and seems to be particularly associated with a severe neuromuscular phenotype. Some variability in the onset and severity of the different symptoms seems to be, even if almost all pediatric cases reported in the literature have a negative prognosis, with quickly progressive worsening of cardiac and respiratory features until exitus, usually occurring within the second to third decade of life (Tab. I). Muscle pathology shows disrupted Z-disks, disorganization of sarcomeric structures, cytosolic aggregated proteins and ectopic accumulation of various myofibrillar proteins and organelles, sign of protein quality control (PQC) and proteolytic systems dysfunction⁹. Studies on a zebrafish model of BAG3 P209L¹⁰ demonstrated a relation between mutated BAG3, protein aggregates and autophagy (a degradation mechanism for damaged proteins in older cells) impairment. However, these studies revealed that mutated Bag3 maintained its function and did not cause protein aggregation but only myofibrillar disruption. Moreover, they demonstrated that aggregate formation was due to the gradual reduction of BAG3 availability caused by itself trapping inside the aggregate. Dysfunctional autophagy was reported in dilated cardiomyopathy due to BAG3 p.Pro209Leu mutation in patient cardiac tissue which demonstrated an increase in autophagy and mitophagy markers¹¹.

The clinical phenotype in our patient is characterized by early onset HOC, early multiple contractures with rigid spine, non-severe proximal and distal weakness, severe axonal motor sensory neuropathy and severe progressive respiratory failure. Symptoms started with HOC at the age of 4 and the evolution of the clinical picture was initially relatively slow. A similar early onset of HOC is reported in other cases, while sometimes heart symptoms appear later, in the adolescence. In BAG3 mutated cases cardiomyopathy can be isolated and progressive and often leads to heart transplantation before the onset of other symptoms. Currently, at the age of 16, in our patient the

cardiological picture shows stability of concentric ventricular hypertrophy with preserved global function. The role played by the treatment with idebenone is probably negligible and the involvement of oxidative metabolism in BAG3-related MFM has not been demonstrated to date. However, a favorable effect on a possible secondary mitochondrial dysfunction cannot be excluded. A possible explanation of the pathologic mechanisms underlying dilated cardiomyopathy in BAG3-related MFM is reported in a recent study of Mc Dermott-Roe et al.¹². Their data imply a pathologic mechanism in which BAG3-RH improperly engages HSC/HSP70: this impairs the formation of multimeric chaperone complexes required for essential protein quality control including, but likely not limited to, myofibrillar maintenance. The observation that fiber disorganization was only apparent when cells were forced to use autophagy suggests that BAG3 variant expressivity is influenced by age-related dynamics in protein quality control subsystem usage. This provides a potential explanation for the delayed onset of BAG3-associated dilated cardiomyopathy and heart failure, often characterized by an aggressive clinical course. Moreover, male sex, low left ventricular EF (< 50%) and increased left ventricular end-diastolic diameter at first evaluation seem associated with an adverse prognosis during follow-up¹³. At 11 years our patient had acute respiratory failure and he began NIV. The worsening of the respiratory function is reported as an early symptom also in the other cases, and BAG3 myopathy has been demonstrated to meet pathologic criteria for hereditary myopathy with early respiratory failure (HMERF), an adult-onset autosomal-dominant myopathy, which typically presents with respiratory muscle weakness in patients who are still ambulant¹³. On the other hand, in BAG3-related MFM multiple contractures and rigid spine may also be present early on and usually worsen with time. These findings may justify the restrictive respiratory failure that is reported in some cases as early onset symptom. Limb muscle weakness was symmetric and mild in our patient, more evident proximally, with mild deficit in distal districts, and unlike other reported cases, ambulation was still preserved with support at the follow-up, while scoliosis and respiratory involvement were severe.

In conclusion, it is important to hypothesize a neuromuscular disorder caused by BAG3 mutations in the presence of early onset HOC (first decade of life) and/or peripheral neuropathy and MFM for a correct diagnosis and to monitor cardiac and respiratory functions, which have usually a bad prognosis. Our case provides further evidence of progressive multisystem clinical involvement of BAG3-related MFM. Natural history studies of BAG3-related MFM are necessary especially in case pharmacological treatments are to be identified, for example com-

Table I. Cases reports in the literature.

Reference	Age at onset (years)	Features at onset	Cardiomyopathy	Contractures	Weakness	Peripheral neuropathy	Respiratory failure	Outcome	BAG3 mutation
Odgerel et al., 2010	5	Not reported	Restrictive-hypertrophic	Not reported	Yes	Axonal neuropathy	Yes	Sudden death at 9 years	P209L
Odgerel et al., 2010	12	Not reported	Restrictive-hypertrophic heart transplantation	Not reported	Yes	Axonal neuropathy	Yes	Not ambulant	Pro209L
Odgerel et al., 2010	12	pes cavus, weakness, cardiopathy	Restrictive hypertrophic	Scoliosis and rigide spine	Distal weakness and neck weakness	Axonal neuropathy	Yes	Death at 20 years	P209L de novo
Odgerel et al., 2010	5	Gait disturbance	Restrictive-hypertrophic heart transplantation	Not reported	Proximal weakness	Axonal neuropathy	Yes	Death at 15 years	P209L de novo
Lee HC et al., 2012	6	Gait disturbance	Restrictive hypertrophic	Multiple contractures and rigide spine	Mild proximal weakness	Axonal neuropathy	Not reported	Ambulant at 12 years	P209L de novo
D'avia et al., 2016	11	Contractures	Hypertrophic and arrhythmia	Rigide spine	Proximal weakness	Axonal neuropathy	Yes	Not ambulant	P209L de novo
Selcen et al., 2009	Toddler	Toe walker	restrictive heart transplant	Toe walker	Severe weakness	Not reported	Yes	not reported	Not reported
Selcen et al., 2009	13	Scoliosis rigide spine, fatigability	hypertrophic	Scoliosis and rigide spine	Distal and proximal weakness	Axonal demyelinating neuropathy	Yes	not reported	Not reported
Selcen et al., 2009	Toddler	Toe walker	Restrictive	Scoliosis, rigide spine and toe walker	Progressive proximal weakness	Not reported	Yes	Death at 13 years	Not reported
Jaffer et al., 2012	Toddler	Toe walker	Restrictive-heart transplantation	Multiple contractures and rigide spine	Distal and proximal weakness	Axonal neuropathy	Not reported	Not ambulant	P209L de novo
Jaffer et al., 2012	Toddler	Toe walker	Restrictive	Multiple contractures, scoliosis and rigide spine	Distal and proximal weakness	Axonal neuropathy	Yes	Ambulant at 13,5 years	P209L de novo
Kostera Pruszczyk et al., 2015	12	Toe walker and foot deformity	Restrictive (subclinical) long QT	Rigide spine and multiple contractures	Subclinical weakness	Axonal demyelinating neuropathy	No	Ambulant at 15 years	Not reported

Table 1. Cases reports in the literature.

Reference	Age at onset (years)	Features at onset	Cardiomyopathy	Contractures	Weakness	Peripheral neuropathy	Respiratory failure	Outcome	BAG3 mutation
Konersman et al., 2015	Not reported	Cardiopathy	restrictive heart transplant	Rigide spine	Severe weakness	Sensory-motor neuropathy	Not reported	Not ambulant	P209L de novo
Seung Ju Kim et al., 2018	11	Gait disturbance and rigid spine	No	Rigide spine and multiple contractures	not reported	Axonal neuropathy	yes	Not reported	P209L de novo
Noury et al., 2018	Not reported	Not reported	No	Rigide spine	not reported	Axonal sensory-motor neuropathy	Not reported	Not reported	Not reported
Current report	4	Cardiopathy	Restrictive-hypertrophic	Multiple contractures and rigide spine	Proximal weakness and mild distal weakness	Axonal neuropathy	Yes	Ambulant with support	P209L de novo

pounds resulted able to removing protein aggregates such as metformin ¹⁴.

Ethical consideration

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Acknowledgement

None.

Funding

None.

Conflict of interest

None of the authors has any conflict of interest to disclose.

Author contributions

GS wrote the manuscript. AP and MG visited the patient and collected clinical informations. AP coordinated the contributes of each co-Author to the paper. MLV performed muscle biopsy histological and immunofluorescence analyses. MC performed muscle ultrastructural analyses. AT and VN performed NGS genetic analyses. LR performed cardiological assessment.

References

- Selcen D, Muntoni F, Burton BK, et al. Mutation in BAG3 causes severe dominant childhood muscular dystrophy. *Ann Neurol* 2009;65:83-89. <https://doi.org/10.1002/ana.21553>
- Zagaa Odgerel, Sarkozy A, Lee H-S, et al. Inheritance patterns and phenotypic features of myofibrillar myopathy associated with a BAG3 mutation *Neuromuscul Disord* 2010;20:438-442 <https://doi.org/10.1016/j.nmd.2010.05.004>
- D'Avila F, Meregalli M, Lupoli S, et al. Exome sequencing identifies variants in two genes encoding the LIM-proteins NRAP and FHL1 in an Italian patient with BAG3 myofibrillar myopathy. *J Muscle Res Cell Motil* 2016;37:101-115. <https://doi.org/10.1007/s10974-016-9451-7>
- Noury J-B, Maisonobe T, Richard P, et al. Rigid spine syndrome associated with sensory-motor axonal neuropathy resembling Charcot-Marie-Tooth disease is characteristic of bcl-2-associated atnogene-3 gene mutations even without cardiac involvement. *Muscle Nerve* 2018;57:330-334. <https://doi.org/10.1002/mus.25631>
- Konersman CG, Bordini BJ, Scharer G, et al. BAG3 myofibrillar myopathy presenting with cardiomyopathy *Neuromuscular Disorders* 2015;25:418-422. <https://doi.org/10.1016/j.nmd.2015.01.009>

- ⁶ Fu J, Ma M, Song J, et al. BAG3 p.Pro209Ser mutation identified in a Chinese family with Charcot-Marie-Tooth disease. *J Neurol* 2020;267. <https://doi.org/10.1007/s00415-019-09680-8>
- ⁷ Selcen D. Myofibrillar myopathies. *Neuromuscul Disord* 2011;21:161-171. <https://doi.org/10.1016/j.nmd.2010.12.007>
- ⁸ Savarese M, Di Fruscio G, Mutarelli M, et al. MotorPlex provides accurate variant detection across large muscle genes both in single myopathic patients and in pools of DNA samples. *Acta Neuropathol Commun* 2014;2:100. <https://doi.org/10.1186/s40478-014-0100-3>
- ⁹ Ruparelia AA, Oorschot V, Vaz R, et al. Zebrafish models of BAG3 myofibrillar myopathy suggest a toxic gain of function leading to BAG3 insufficiency. *Acta Neuropathol* 2014;128:821-833. <https://doi.org/10.1007/s00401-014-1344-5>
- ¹⁰ Schänzer A, Rupp S, Graf S, et al. Dysregulated autophagy in restrictive cardiomyopathy due to Pro209Leu mutation in BAG3. *Mol Genet Metab* 2018;123:388-399. <https://doi.org/10.1016/j.ymgme.2018.01.001>
- ¹¹ McDermott-Roe C, Lv W, Maximova T, et al. Investigation of a dilated cardiomyopathy-associated variant in BAG3 using genome-edited iPSC-derived cardiomyocytes. *JCI Insight* 2019;4:e128799. <https://doi.org/10.1172/jci.insight.128799>
- ¹² Domínguez F, Cuenca S, Bilinska Z, et al. Dilated Cardiomyopathy Due to BLC2-Associated Athanogene 3 (BAG3) Mutations. *J Am Coll Cardiol* 2018;72:2471-2481. <https://doi.org/10.1016/j.jacc.2018.08.2181>
- ¹³ Pfeffer G, Povitz M. Respiratory management of patients with neuromuscular disease: current perspectives. *Degener Neurol Neuromuscul Dis* 2016;6:111-118. <https://doi.org/10.2147/DNND.S87323>
- ¹⁴ Ruparelia AA, Mckaige EA, Williams C, et al. Metformin rescues muscle function in BAG3myofibrillar myopathy models. *Autophagy* 2020. <https://doi.org/10.1080/15548627.2020.1833500> [Epub Ahead of Print]